

FUNCTIONAL EXPRESSION OF ENaC IN 293T

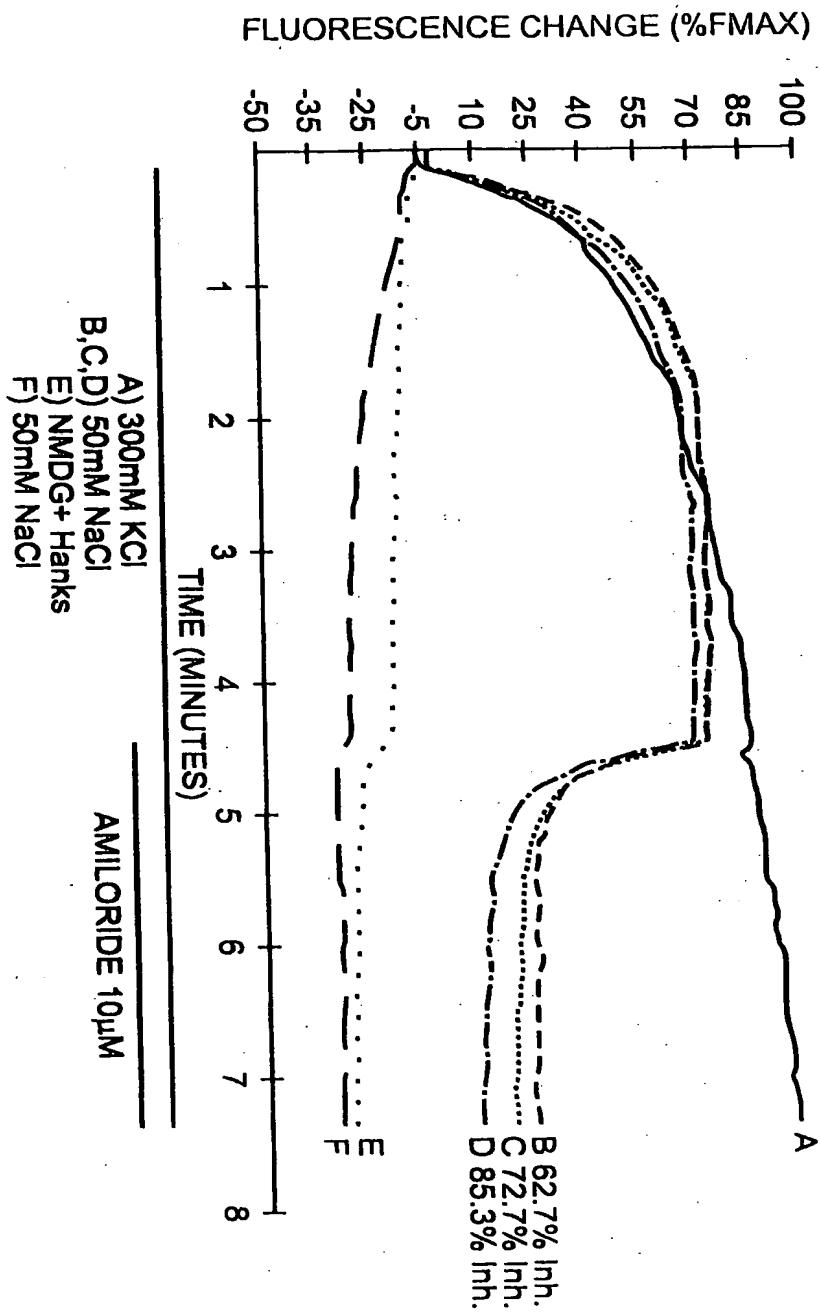
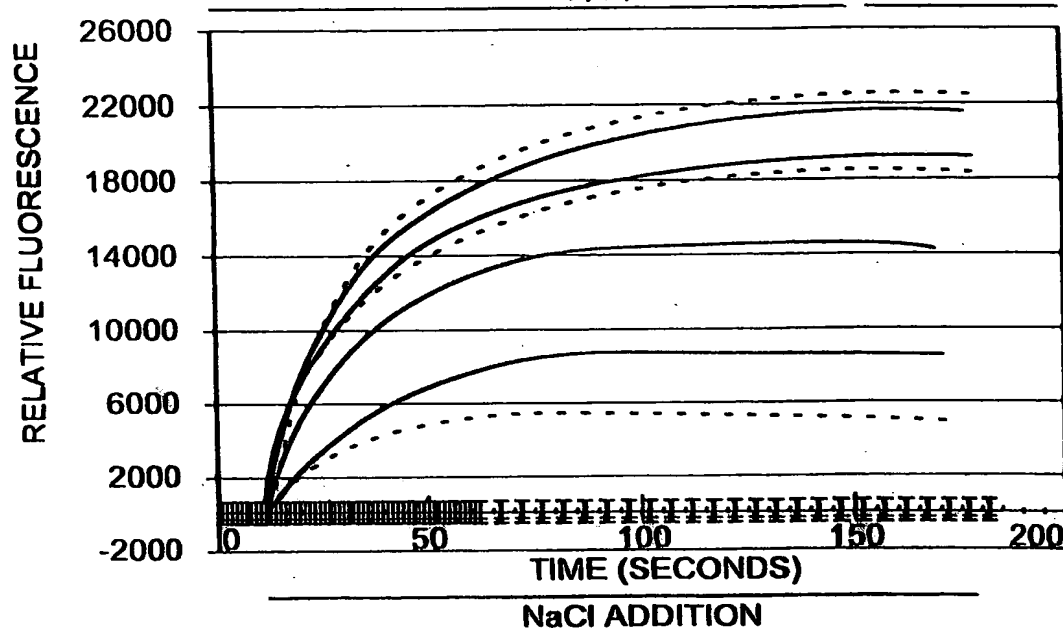


FIGURE 1

NaCl DOSE RESPONSE RELATIONSHIP IN HEK293T EXPRESSING
hENaC α , β , γ



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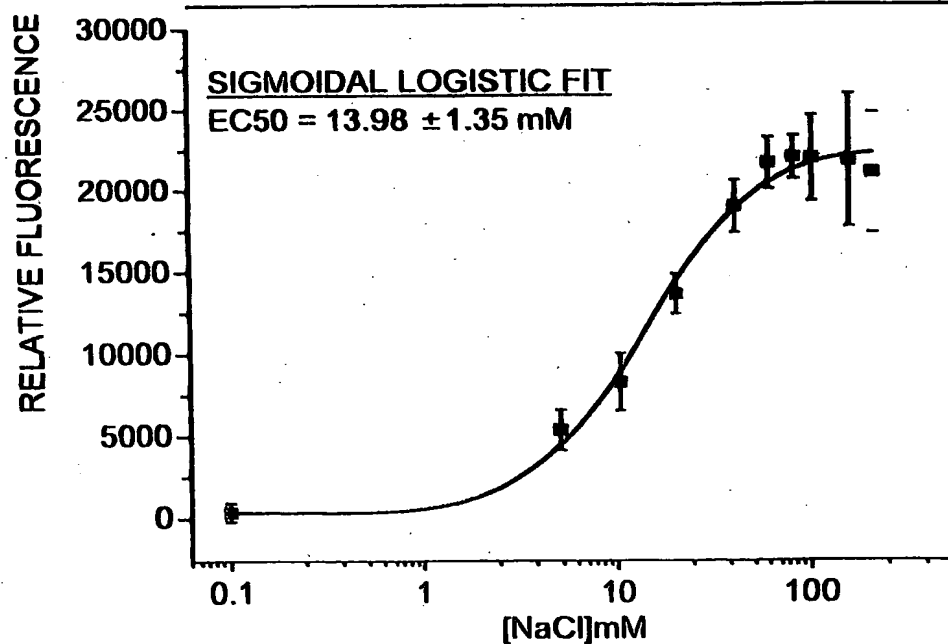


FIGURE 2

HEK293T EXPRESSING HENAC $\alpha_2\beta_1$

MULTIPLE WELL AVERAGE

FLUORESCENCE CHANGE (COUNTS)

TIME (MINUTES)

50 mM NaCl

AMILORIDE



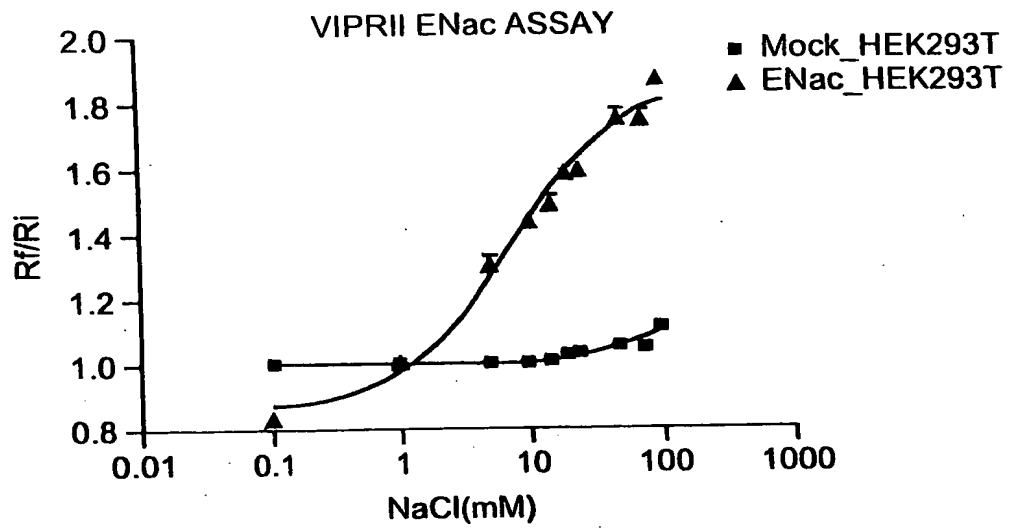


FIGURE 4

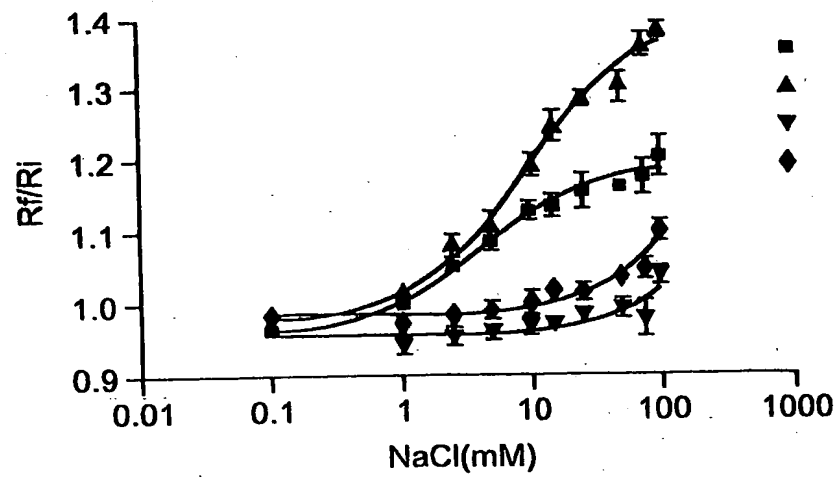


FIGURE 5

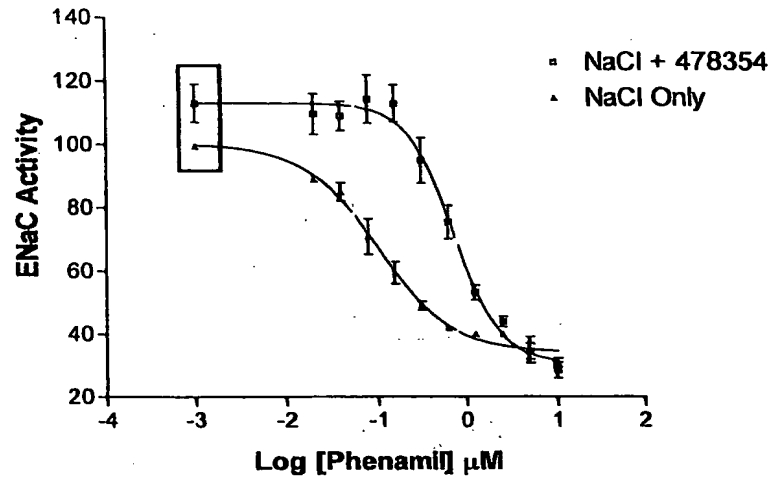
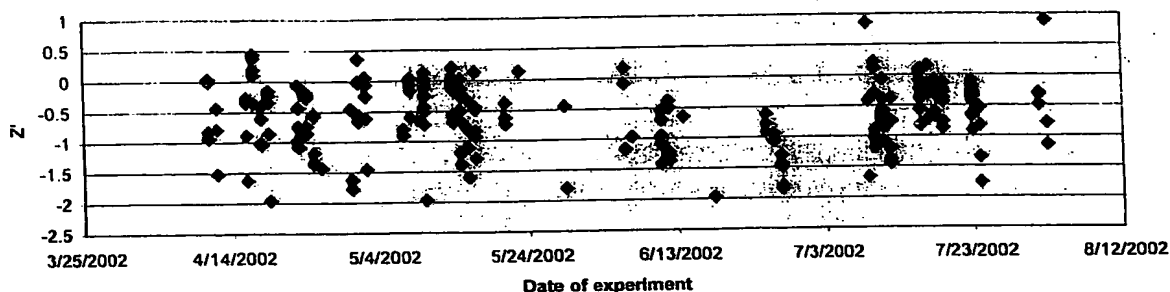


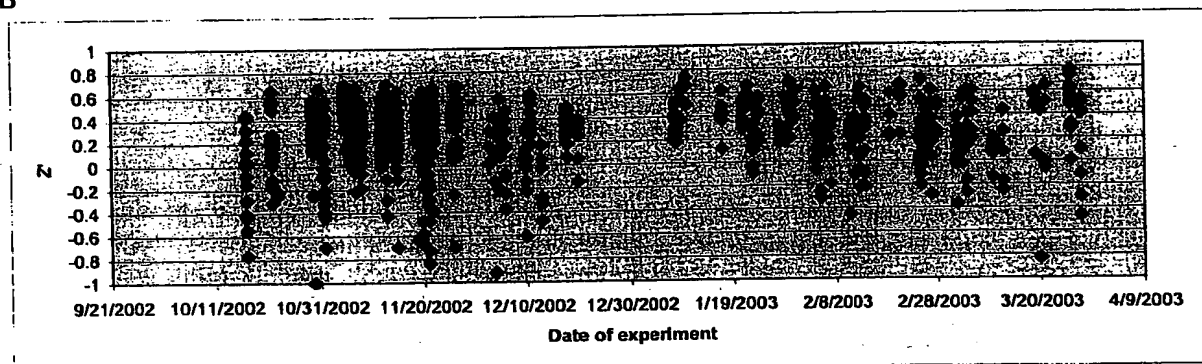
Figure 6: Effect of increasing concentrations of phenamil on ENaC activity. Blue trace: inhibition of ENaC activity by phenamil. Red trace: inhibition of ENaC activity by phenamil in the presence of 100 μM 478354, an ENaC enhancer. Black box contains data showing 478354 effect in the absence of phenamil. Yellow box contains data showing enhanced 478354 effects by the presence of increasing concentration of phenamil.

FIGURE 6

A



B



| Z' (range) | # of experiments |
|------------|------------------|
| >0.5 | 153 |
| 0.2 to 0.5 | 417 |
| 0 to 0.2 | 149 |
| <0 | 116 |

Figure 7: *A.* Distribution of Z' in the absence of phenamil. Z' is define as: $1 - ((3 \times \text{standard deviation of ENaC response} + 3 \times \text{standard deviation of ENaC response in the presence of 478354}) / (\text{mean ENaC activity in the presence of 478354} - \text{mean ENaC activity}))$. Most Z' values are less than 0 indicating that, when used as the high control, 478354 cannot provide a meanful assay window. *B.* Distribution of Z' in the presence of 0.5 μM phenamil. Z' is define as: $1 - ((3 \times \text{standard deviation of ENaC response} + 3 \times \text{standard deviation of ENaC response in the presence of 478354}) / (\text{mean ENaC activity in the presence of 478354} - \text{mean ENaC activity}))$. Most Z' values are > 0 and ≤ 1 indicating that, when used as the high control, 478354 can provides a meaningful assay window in the presence of phenamil.

FIGURE 7

FIGURE 8

$$\% \text{ Enhancement Factor} = [(A - A_o) / (B - B_o)] \times 100\%$$

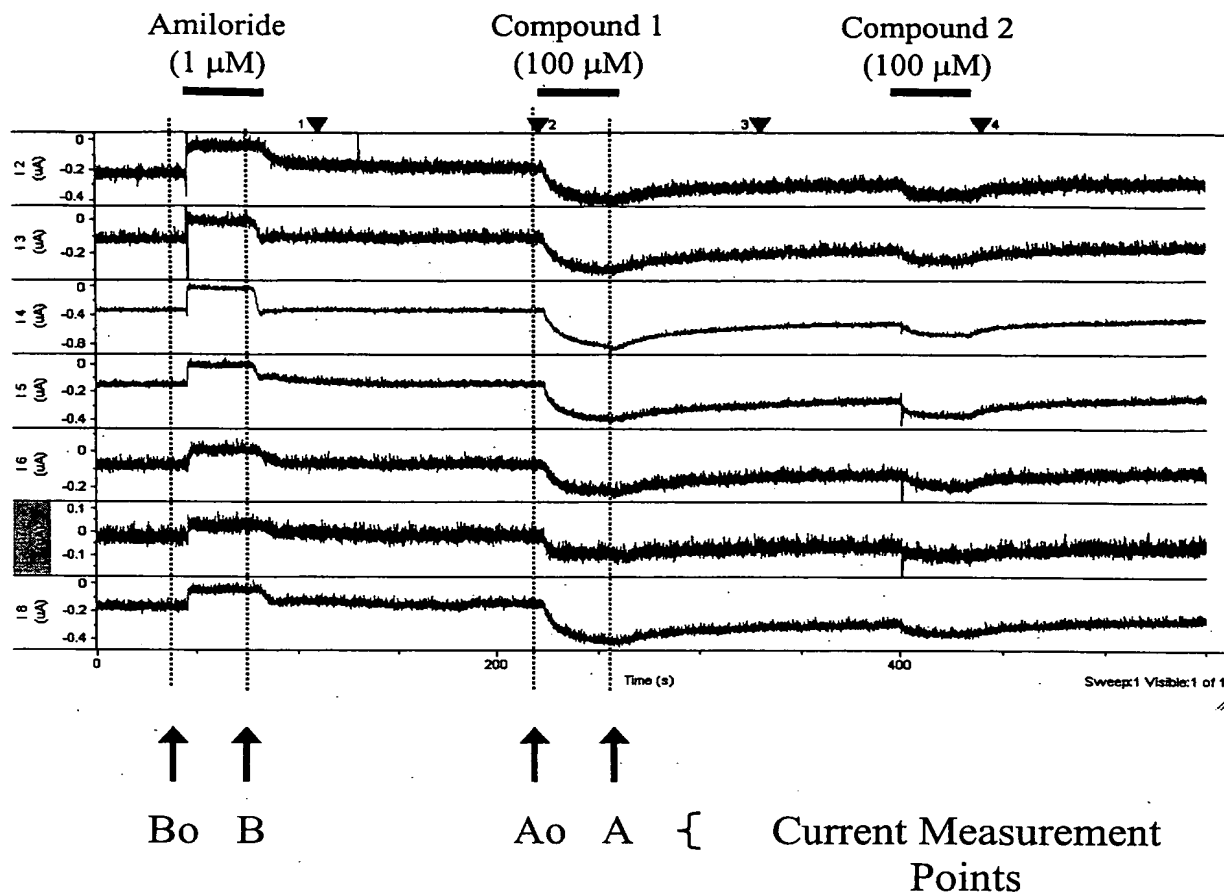
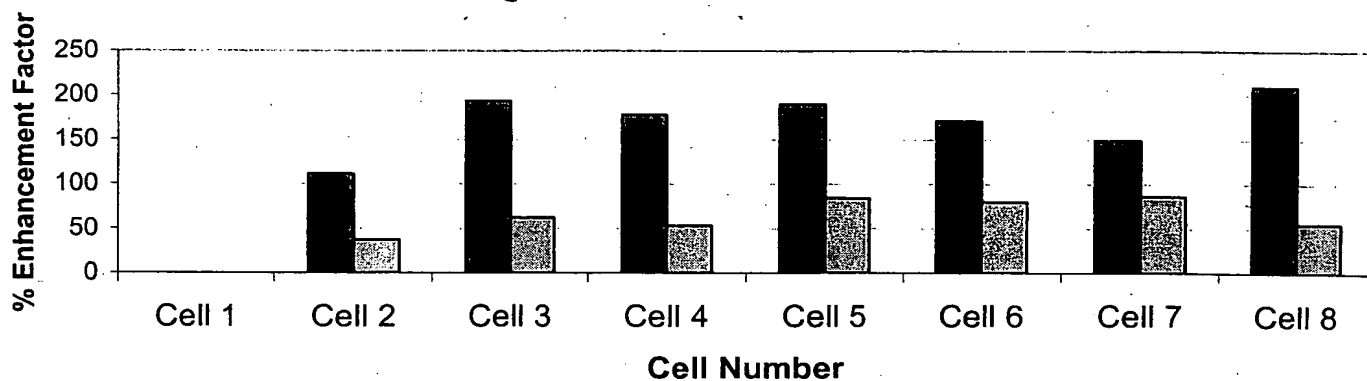


FIGURE 9

Quantitation of ENaC Enhancement



Compound 1 = 171.8 \pm 32.7 %
 Compound 2 = 65.4 \pm 18.5 %

FIGURE 10

Example Uninjected Oocytes

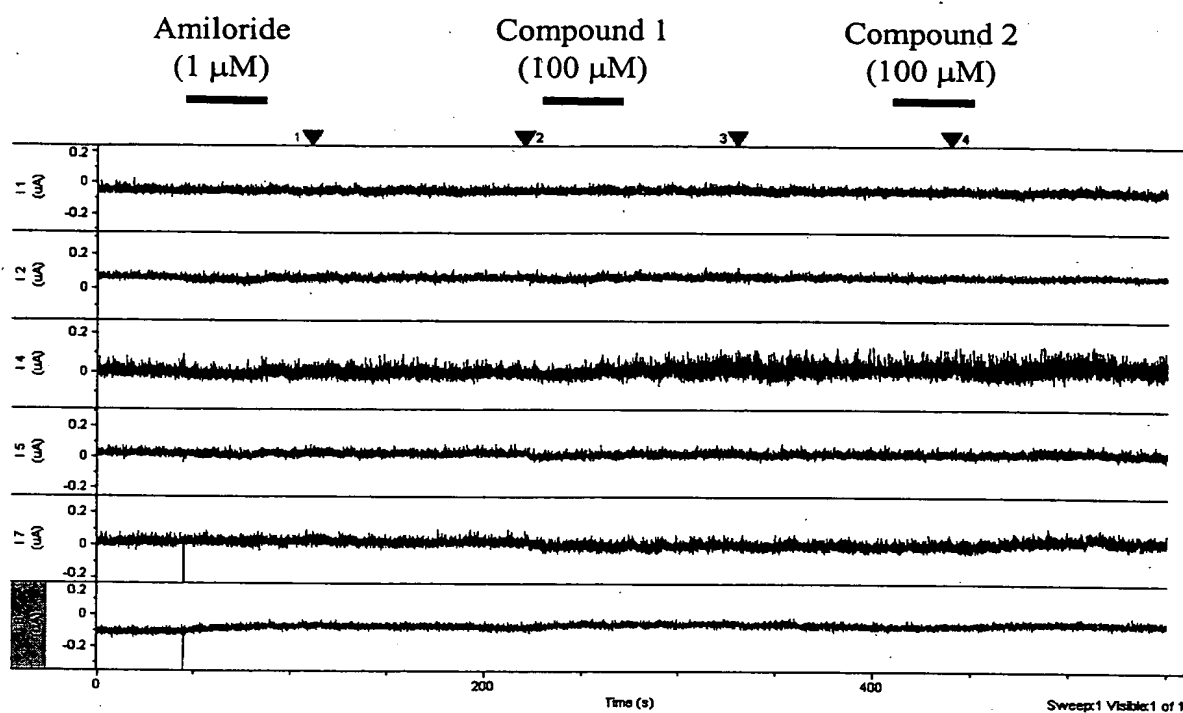
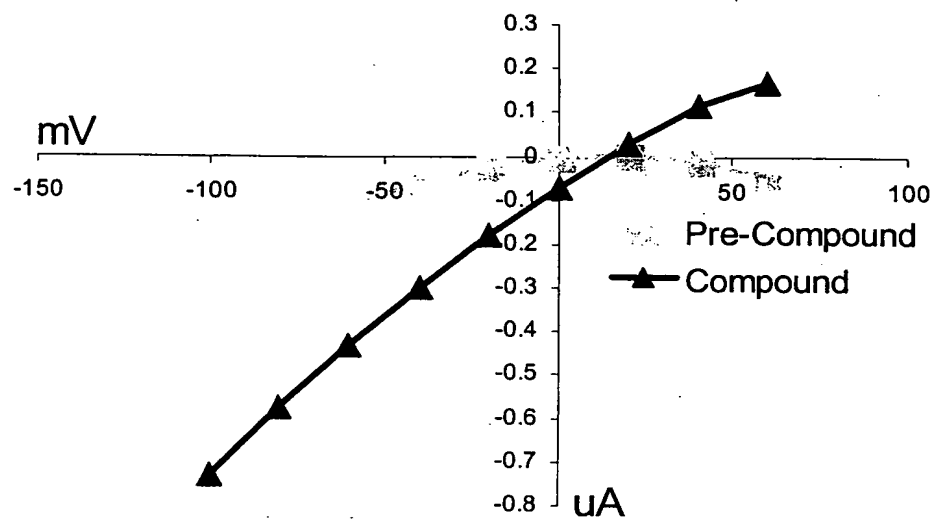


FIGURE 11

Example I/V Curves

A) Injected eggs



B) Uninjected eggs

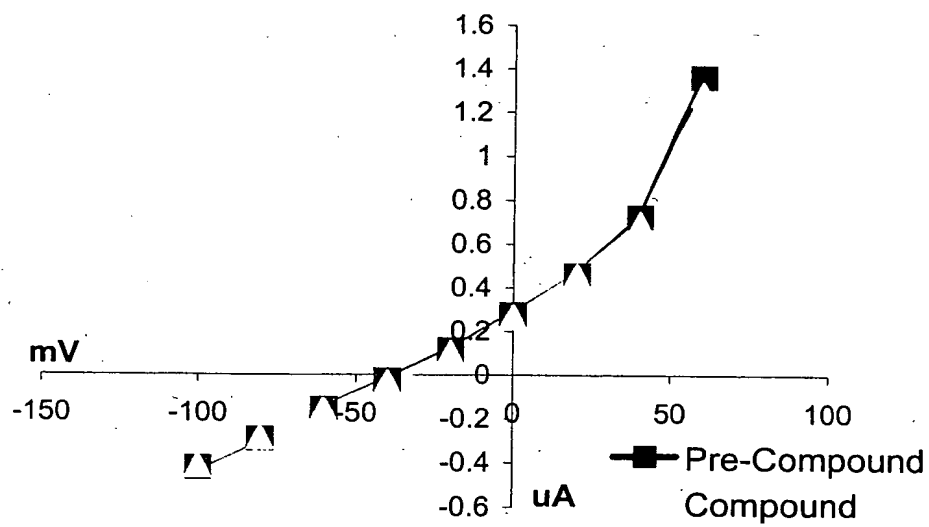


FIGURE 12

Example Amloride Competition

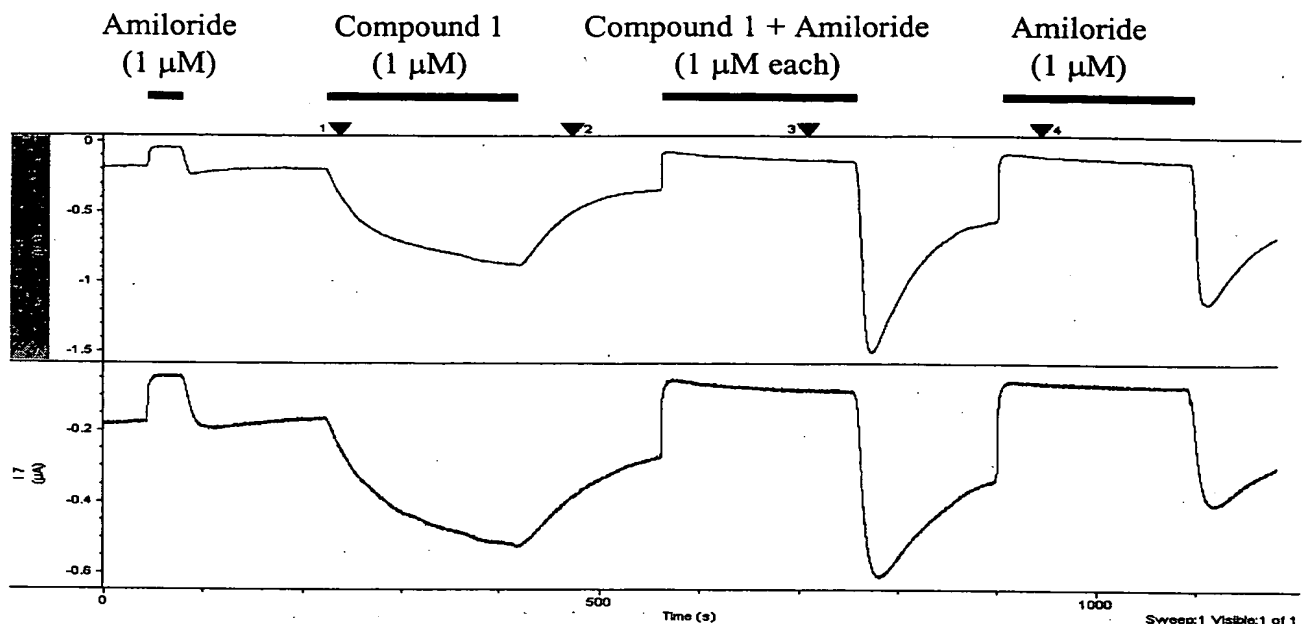
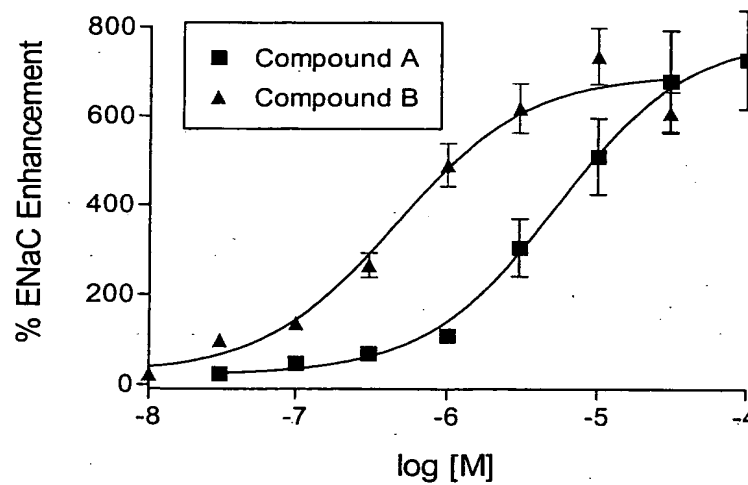


FIGURE 13

Example Dose Response Curves



| [M] | Compound A | Compound B |
|------|-------------|-------------|
| EC50 | 5.3980e-006 | 4.6760e-007 |

FIGURE 14

ENaC Oocyte Assay Flowchart

